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Bacteriology of Butter

IV. Bacteriological Studies on Surface Taint Butter

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CONTENTS

SUMMARY

1. The surface taint butter examined often contained large numbers of bacteria, as determined by the plate method, and with some of the samples the counts were very high; with a few samples the counts were comparatively low. The counts were higher on the surface portion of a sample than on the interior portion in all but 1 of 20 comparisons.

2. The surface taint butter examined usually contained large numbers of yeasts, with the surface portion of a sample commonly containing a larger number than the interior portion; the butter showed some mold counts that were high and a few that were surprisingly high, the counts on the surface portion generally being higher than on the interior portion.

3. The general types of bacteria found in surface taint butter by picking colonies into litmus milk from beef infusion agar plates were essentially the same as those found in any lot of butter containing considerable numbers of organisms.

4. Surface taint could not be produced in butter by inoculating a normal product, either salted or unsalted, with surface taint butter but could be developed by inoculating the defective butter into pasteurized cream and churning the cream; from 2 to 4 days were required for the surface taint to develop at 15.6° C. (60° F.) and from 7 to 10 days at 5° C. (41° F.).

5. Surface taint sometimes developed in commercial butter held at temperatures very favorable for bacterial growth; in most cases the butter that developed surface taint was unsalted or had a low salt content.

6. Excessive numbers of organisms were found when either experimental or commercial surface taint butter was examined microscopically.

7. The organisms which predominated on beef infusion agar plates poured with surface taint butter did not produce surface taint when inoculated into pasteurized cream and the cream churned.

8. An organism capable of producing surface taint when inoculated into pasteurized cream and the cream churned was first isolated from a sample of Canadian butter by plating on beef infusion agar and picking colonies into litmus milk; at the time of the appearance of surface taint in experimental butter the numbers of organisms per milliliter, as determined by the plate method, were comparatively small. The organism was believed to be an undescribed species and was tentatively designated *Achromobacter putrefaciens.*

9. By the use of an enrichment method consisting of inoculating butter into litmus milk, holding this at 5° C. (41° F.) and then plating on beef infusion agar and picking colonies into litmus milk, *A. putrefaciens* was secured from five additional samples of surface taint butter, three from Canada and two from the United States. From a considerable number of surface taint samples *A. putrefaciens* could not be secured.

10. Organisms, other than *A. putrefaciens,* which would produce surface taint were isolated from a number of samples of surface taint butter. These organisms were always found in small numbers.

11. Organisms which would produce surface taint were isolated from a total of 17 samples of commercial surface taint butter, 6 from Canada and 11 from the United States.

12. Organisms capable of producing surface taint could not be isolated from a considerable number of samples of surface taint butter, although with some of the samples the defect could be carried through a series of experimental churnings by using defective butter to inoculate the cream.

13. The organisms which would produce surface taint were greatly restrained by the use of medium salt percentages or butter culture in the making of butter. *A. putrefaciens* failed to grow in skimmilk acidified with lactic acid to 0.30 or 0.31 percent but did develop when the milk was acidified to 0.27, 0.28 or 0.29 percent.

14. In the trials carried out with *Ps. fluorescens* by inoculating pasteurized cream and churning the cream, rancidity regularly developed.

15. There appeared to be rather distinct variations in the samples of butter sent to the laboratory as examples of surface taint.

Bacteriology of Butter

IV. Bacteriological Studies on Surface Taint Butter

By H. A. Derby and B. W. Hammer

During recent years a butter defect has been encountered so frequently that it has been differentiated from other types of deterioration and characterized as a definite abnormality under the name, surface taint. Undoubtedly all butter judges do not have exactly the same idea of this defect, and a piece of butter may be considered, by one judge, to show surface taint while another describes the condition differently. The same situation, however, exists with defects that have been studied for many years and the differences of opinion do not prevent these defects from being recognized as definite types of deterioration. Typical surface taint is easily recognizable by butter judges experienced with it, and only when it is present to a very slight degree or in combination with another defect is its detection likely to be difficult.

STATEMENT OF PROBLEM

The work herein reported was undertaken with the object of studying (a) the cause of surface taint and (b) the factors influencing its development.

Because of the studies already reported and the general nature of the odor and flavor of surface taint butter, it seemed probable that (a) the defect was due to microorganisms, and (b) the general type of decomposition was one involving the protein.

CHARACTERS OF SURFACE TAINT BUTTER

Surface taint in butter is a defect in which the odor and flavor more or less definitely suggest putrefaction. It is first evident at the surface and then gradually penetrates to the center.

The defect is essentially one that develops after the butter is made. At comparatively high holding temperatures it may develop rather quickly and be noticeable in butter a few days after manufacture, while at about 5° C. (41° F.) 10 days or more may be required for it to be evident. Very often holding a small piece of butter overnight at room temperature so intensifies the odor and flavor that a sample which was questionable before the holding becomes a definite example. Reports indicate that in some instances butter may be stored for consid-

erable periods, and then develop the condition when it warms up during the handling in retail channels. Butter which shows the defect primarily at the surface quickly develops it at newly cut surfaces when the temperature is favorable.

The continued holding of surface taint butter at a temperature suitable for the growth of organisms ordinarily results in the development of an extremely objectionable odor and flavor. The surface taint may entirely disappear; presumably the products responsible for it are either changed or are overshadowed by other decomposition products. Pronounced rancidity sometimes develops in surface taint butter that has been held at comparatively high temperatures.

OCCURRENCE OF SURFACE TAINT IN BUTTER

Surface taint was apparently first recognized as a definite butter defect in western Canada in 1919. Since then it has been found in other parts of the Dominion. Essentially the same defect has been reported from a number of countries under a variety of names; examples of these are the Limburger cheese flavor of butter in the United States, the putrid flavor of butter in Denmark, the foetid odor of butter in New Zealand, and the disagreeable aroma of butter in Australia.

The defect occurs in butter made from good, as well as from poor, raw material. Occasionally churnings of butter that are expected to be of a high quality develop surface taint very rapidly without any reason being evident in the quality of the cream or in the methods of manufacture. Surface taint may appear in one of the churnings at a plant, be absent from a number, and then recur.

Most lots of surface taint butter have been made from pasteurized cream, and often the pasteurization exposure has been comparatively high, for example, 10 minutes at 76.7° C. (170° F.) in certain Canadian plants. Much of the surface taint butter has a rather low salt content and has been made without the use of butter culture.

HISTORICAL

Gilruth (4), working in New Zealand in 1899, developed a foetid odor in butter by inoculating *Bacillus fluorescens liquefaciens* which was isolated from water. The odor appeared in the inoculated butter in 36 hours when incubated at 18.3° C. (65° F.) and in three days when held in a cold room where the temperature was constantly kept under 7.2° C. (45° F.); inoculated butter kept below 0° C. (32° F.) for a month developed the odor in two days when it was placed at a temperature favorable for bacterial growth. Gilruth pointed out that the

results secured indicate butter may be graded first-class in New Zealand and then be "uneatable" soon after its arrival in London because of the activity of organisms gaining entrance to the butter from the water used for washing.

In 1900 Eckles (3) studied an outbreak of putrid butter in Iowa. The butter possessed a strong disagreeable taste with a putrid smell and was considered unsaleable for table use. The taste, although strong, was not as bad as the odor. When a small portion of the defective butter was added to a flask of sterile milk, the putrid odor became so bad within two or three days that the flask had to be removed from the laboratory. A bacteriological examination of the putrid butter showed a large proportion of the organisms present to be of the liquefying type. Four species capable of producing an objectionable change in milk were isolated. Of the four species, two were found capable of bringing about the putrid condition in butter when inoculated into cream and the cream churned; one of these was *Bacterium fluorescens liquefaciens,* but it did not bring about a condition as objectionable as the other. The creamery encountering the defect was advised to reject all raw material showing any suggestion of the putrid condition, to clean and sterilize all equipment, to pasteurize the skimmilk before returning it and to use a large amount of butter culture. About this time an extended dry period was broken by heavy rains. As a result of some or all of these factors the butter improved, and no further trouble was encountered.

The name surface taint was suggested by Marker (9) because the taint is first noticed at the surface of butter. This investigator examined many churnings of surface taint butter and from the observations made concluded that the reaction of the butter resulting from the neutralization of the cream, etc., makes conditions favorable for surface taint development.

Sadler and Vollum (11) studied surface taint butter received in Vancouver and noted that conditions at the creameries from which the butter came permitted contamination following pasteurization. The water supplies used for washing the butter were not satisfactory, and the brines used for the treatment of liners were grossly contaminated with microorganisms of the Escherichia-Aerobacter type. In the case of one creamery, after the necessary precautions had been taken to prevent contamination of the cream and butter subsequent to pasteurization, the trouble ceased. Some of the organisms isolated in the study were used in experimental churnings by Sadler and Cameron (appendix II, ref. 11). A spore-forming, milk-digesting microorganism in combination with an Escherichia-Aerobacter type gave a condition resembling surface taint when used in low acid cream (neutralized to 0.21 or 0.10 percent), but failed

to give the typical condition. When the combination of microorganisms was used in cream of a higher acidity (neutralized to above 0.30 percent) there was no evidence of any particular deterioration.

Cordes (2) and also Macy (8) found surface taint butter invariably contained large numbers of bacteria and yeasts; Macy believed the defect to be due to a large coccus form in associative action with yeasts.

Hood and White (6) reported that all samples of surface taint butter analyzed during 1926 and 1927 were abnormally high in bacteria and yeasts and contained large numbers of bacteria capable of decomposing curd. Bacterial counts and yeast and mold counts on butter from plants having trouble with surface taint were unusually high; some plants, however, showed both high and low counts, indicating inconsistent sanitary control. Many samples of butter made from unpasteurized cream contained fewer bacteria than surface taint butter. Hood and White produced surface taint with organisms isolated from well water by inoculating them into pasteurized, neutralized cream and churning the cream; the defect was evident in as short a time as two days. Some of the creamery water supplies examined were found to contain large numbers of liquefying bacteria.

The spasmodic occurrence of a defect in New South Wales butter, described as disagreeable aroma, has been studied by Brown (1). The description of the defect agrees rather closely with that of surface taint. This investigator stated that instances have been noted by the government officials in which contamination from insanitary churns, workers and other wooden utensils, and the consequent bacterial action brought about by this condition, have been definitely proved to be at least partly responsible for the aroma. An investigation by one of the officials led to the conclusion that it is not probable the defect commences in the manufactured butter since, in the examination of many samples of the tainted butter, putrefying bacteria were not constantly found in numbers large enough to cause the trouble. This official is of the opinion that the putrescent, curdy material which exudes from the glands of the cream storage vats, and the decomposed buttermilk from crevices in the churns, workers, etc., become incorporated in the butter during manufacture, and there, in the form of minute particles, continue to alter, apparently through the action of enzymes and bacterial byproducts; the odor of decomposition characteristic of affected butter is thus produced. Brown reported that when churns, workers and other wooden appliances receive disinfecting treatments and a thorough cleansing, and in some cases when new equipment is substituted for fat-saturated insanitary articles, the defect disappears.

Shutt (12) noted that all samples of surface taint butter have had their origin at creameries where the water supplies have not been pure and that practically all outbreaks or surface taint have followed periods of heavy rainfall. The water supplies of all creameries investigated were found to be contaminated with large numbers of putrefactive bacteria, chief of which has been *Pseudomonas fluorescens*. Organisms secured from water were found capable of producing surface taint when inoculated into cream and the cream churned; these proved to be *Ps. fluorescens*. Shutt reported surface taint could be controlled by (a) substituting pure water for contaminated, (b) subjecting contaminated water to 87.8° C. (190° F.) for 10 minutes and (c) neutralizing the cream to not less than 0.35 percent acid.

Data secured by MacKay (7) showed that during 1928 and 1929 considerable surface taint was encountered in the provinces of Alberta, Saskatchewan and Manitoba; during 1928 the percentages of the total pounds of butter examined that showed surface taint varied from 1.45 to 3.12 for the different provinces, while during 1929 the percentages ranged from 1.41 to 2.52.

EXPERIMENTAL

BACTERIAL COUNTS AND YEAST AND MOLD COUNTS ON COMMERCIAL SURFACE TAINT BUTTER

Bacterial counts and yeast and mold counts were made on a number of samples of butter that were sent to the laboratory as examples of surface taint. Samples 1 to 20, inclusive, were from Canada and were held in cold storage for some time between the detection of the defect and the microbiological analyses; generous amounts (either 3½ or 7 pounds) were available with all of them. Some of samples 21 to 35, inclusive, were from Canada and some from the United States; the samples were taken soon after the defect was noted and only a small amount of each was available. All of the samples were sent to the laboratory without refrigeration so that temperature conditions during the transportation were often satisfactory for growth. On arrival many of the samples showed a typical surface taint, while a few seemed to have some other odor and flavor defect as the conspicuous one; the opportunity for the growth of organisms during transportation suggests that the odor and flavor may have undergone pronounced changes. All of the samples contained salt.

The bacterial counts obtained by the plate method, using beef infusion agar and an incubation of four days at 21.1° C. (70° F.) are presented in tables I and II; table I gives the results obtained on 20 samples which were large enough so that

TABLE I. BACTERIA IN SURFACE TAINT BUTTER.

Sample no.	Plate method	
	Bacteria per ml.	
	Surface	Interior
1	303,000,000	14,000,000
2	36,000,000	22,400,000
3	137,500,000	18,000,000
4	20,500,000	6,500,000
5	20,000,000	18,200,000
6	65,200,000	29,800,000
7	5,850,000	1,650,000
8	72,000,000	950,000
9	18,900,000	11,900,000
10	1,030,000	565,000
11	28,500,000	2,450,000
12	129,000,000	25,000,000
13	16,000,000	6,000,000
14	19,000,000	7,350,000
15	69,500,000	29,000,000
16	1,940,000	148,000
17	5,600,000	3,200,000
18	9,800,000	2,000,000
19	1,840,000	2,400,000
20	2,100,000	50,000

both a surface and an interior portion could be examined, while table II gives the results obtained on the 15 small samples. The data show that the surface taint butter often contained large numbers of bacteria and that with some of the samples the counts were surprisingly high, while with a few the counts were comparatively low. It should be noted that, as was pointed out in the discussion of the sources and general character of the samples, some of them may have had defects other than surface taint. The count on the surface portion was higher than that on the interior portion in all but 1 of the 20 comparisons and, in some instances, the differences were very large.

The yeast and mold counts were also made by the plate method; whey agar was used, one ml. of a 1 percent solution of tartaric acid being added to each plate just before the agar was poured, and the plates were incubated for four days at 21.1° C. (70° F.).* An attempt was made to count the yeasts and

*Although this procedure is not, at present, one that is commonly used, the studies were begun with it and, accordingly, its use was continued.

TABLE II. BACTERIA IN SURFACE TAINT BUTTER.

Sample no.	Plate method	Sample no.	Plate method
	Bacteria per ml.		Bacteria per ml.
21	9,250,000	29	5,200,000
22	13,000,000	30	1,500,000
23	177,500,000	31	30,000,000
24	11,000	32	18,000,000
25	39,000,000	33	20,000,000
26	445,000	34	14,000,000
27	2,500,000	35	600,000
28	37,000,000		

molds separately, although the differentiation was probably not accurate. The results obtained on both the surface and interior of each of 20 samples are given in table III. The numbers of yeasts were usually high and often very high; the count on the surface portion was higher than that on the interior portion in all but two of the comparisons, and in a number of instances the differences were large. A number of the mold counts were rather high and a few of them were extremely high; in general, the count on the surface portion was higher than that on the interior portion, but with three samples molds were absent at both the surface and interior in the dilutions used, and with two samples the count was higher at the interior than at the surface, the difference being definite in one instance while it was within the limits of experimental error in the other.

TABLE III. YEASTS AND MOLDS IN SURFACE TAINT BUTTER.

Sample no.	Plate method			
	Yeasts per ml.		Molds per ml.	
	Surface	Interior	Surface	Interior
1	108,500	770	0	0
2	3,000,000	965	45	20
3	2,000	940	70	0
4	24,000	62,000	35	20
5	16,000	4,400	20	10
6	2,750	2,400	75	10
7	70,500	54,500	50	0
8	43,000	190	10	5
9	97,500	47,000	1,000	0
10	80	210	10	0
11	170	50	0	0
12	70,000	9,000	1,000	13,000
13	465,000	200,000	28,000	0
14	200,000	153,000	100	0
15	2,000	450	5	0
16	35,000	21,000	0	0
17	1,400	1,100	10	20
18	4,400	2,600	10	0
19	104,000	80	10	0
20	320,000	23,000	10	10

The excessive numbers of microorganisms commonly found in surface taint butter are in agreement with the findings of various investigators. The numbers suggest that considerable growth must have occurred because such high counts would not be expected in butter made from pasteurized cream unless growth had taken place, regardless of the quality of the raw material or the manufacturing methods. It must be recognized, however, that in butter showing various defects and also in certain samples without any conspicuous abnormality, excessive numbers of organisms are common and that the surface portions frequently show higher counts than the interior portions.

In connection with three of the surface taint samples, normal samples of butter representing the churning just before or just

after the one developing the defect were examined for the numbers of bacteria; the results secured are given in table IV. In two instances the surface taint sample had a definitely higher bacterial count than the sample or samples with which it was compared, while in one instance the bacterial count of the surface taint sample was between those of the normal samples with which it was compared.

TABLE IV. BACTERIAL COUNT OF SURFACE TAINT BUTTER COMPARED WITH THAT OF NORMAL BUTTER REPRESENTING THE CHURNING JUST BEFORE OR JUST AFTER THE SURFACE TAINT CHURNING.

Comparison no.		Plate method		
		Bacteria per ml.		
		Churning before that developing surface taint	Surface taint sample	Churning after that developing surface taint
1	Surface	20,000	1,030,000	
	Interior	15,000	565,000	
2	Surface	54,000,000	28,500,000	4,900,000
	Interior	5,800,000	2,450,000	1,500,000
3		28,000,000	177,500,000	17,500,000

GENERAL TYPES OF BACTERIA IN SURFACE TAINT BUTTER

A study was made of the general types of bacteria present in surface taint butter (with some samples both the surface and interior were studied) by picking 25 contiguous colonies into litmus milk from a beef infusion agar plate; these milk cultures were incubated at room temperature for 14 days and then classified, according to the changes produced, into acid coagulators, acid non-coagulators, proteolyzers, alkali formers and inert. The results obtained on 15 samples are given in table V. In general large percentages of acid formers (coagulators or non-coagulators) or inert forms were found so that the proteolyzers and alkali-formers were not especially conspicuous. When both a surface and an interior portion from a sample were examined the general types of organisms in the two parts were essentially the same. The odors produced in milk by most of the cultures picked were not particularly objectionable and, commonly, even the proteolyzers failed to produce in milk a putrefactive odor suggestive of surface taint butter.

The bacteria secured from surface taint butter were of various morphologic types. Micrococci were especially conspicuous, while rods, particularly non-spore-forming, gram-negative types, and streptococci were regularly present. There was considerable variation in the predominating type

TABLE V. GENERAL TYPES OF BACTERIA PRESENT IN SURFACE TAINT BUTTER.

Sample no.		Percentages of				
		Acid		Proteolyzers	Alkali formers	Inert
		Coagulators	Non-coagulators			
1	Surface	8	64			28
	Interior		64	4		32
2	Surface	4	56		4	36
	Interior	4	52			44
3	Surface		80			20
	Interior		88			12
4	Surface		44		24	32
	Interior		68	4	8	20
5	Surface		52	28	4	16
	Interior		64	20		16
6	Surface		40	4	4	52
	Interior		64	8	4	24
7	Surface		68			32
	Interior		76			24
8	Surface		88			12
	Interior		96	4		
9	Surface		24		16	60
	Interior		24	4	8	64
10	Surface		32		4	64
11	Surface	28	24	12	20	16
12	Surface	8	56	16	12	8
	Interior	40	40	20		
21		56		4	16	24
22		48	20		4	28
23		40	8	36	4	12

from one sample to another. The general types of bacteria found in surface taint butter by picking colonies into litmus milk from beef infusion agar plates were essentially the same as those that are found in any lot of butter showing considerable numbers of organisms. This suggests that there is no conspicuous change in the bacterial flora with the development of surface taint in butter.

PRELIMINARY ATTEMPTS TO PRODUCE SURFACE TAINT

The initial attempts to produce surface taint involved the direct inoculation of surface taint butter into a normal product that was either unsalted or had a low salt content. The procedures used varied from inoculation with a needle to the working in of the inoculating material with a sterile spatula. Although various holding temperatures at which surface taint

develops under practical conditions were employed, the trials were regularly unsuccessful. Occasionally there would be some suggestion of surface taint in the inoculated butter, but this did not increase on standing at room temperature, as is usually the case when surface taint is present in butter to only a slight extent.

The addition of surface taint butter to cream shortly before churning was then used as an inoculation procedure. The cream was pasteurized at 76.7° C. (170° F.) for 10 minutes, cooled and inoculated with a small amount of defective butter (the butter being melted at as low a temperature as possible) by distributing the butter over the surface of the cream and shaking at once. After it had been held at 15.6° C. (60° F.) for from 2 to 12 hours, the cream was churned in a glass jar, using a shaking machine. The resulting butter was washed twice with sterile water, first rinsing the granules with a small amount of water and then shaking the granules in a larger amount. The washed butter was worked in a sterile container with a butter paddle; some lots were salted lightly, while others were unsalted. The butter made with this procedure commonly developed a condition which was considered to be typical surface taint. As a rule from 2 to 3 days were required at 15.6° C. (60° F.) and from 7 to 10 days at 5° C. (41° F.). Experimentally produced surface taint butter also rather regularly produced the defect in butter made from pasteurized cream into which it was inoculated. In this way the defect was sometimes produced down through a series of churnings. Eventually, however, some other off condition would overshadow the surface taint and become the prominent defect in subsequent churnings. One of the defects that commonly overshadowed the surface taint was rancidity, and in some instances the rancidity was very pronounced.

Occasionally samples of butter were received that were considered, by the sender, to show surface taint but which had an odor and flavor definitely suggesting very poor raw material. Instead of the characteristic surface taint odor and flavor, a stale, cheesy odor and flavor suggestive of old, high-acid cream were present. The samples often showed only a few thousand bacteria per milliliter by the plate method. Attempts to produce surface taint butter by inoculating the samples into pasteurized cream and then churning the cream were regularly unsuccessful, and often the butter secured by this procedure kept very well. These results definitely suggest that the odor and flavor of the butter were due to poor material rather than to bacterial action following the manufacture of butter.

PRODUCTION OF SURFACE TAINT BY HOLDING SAMPLES OF COMMERCIAL BUTTER

The keeping qualities of a large number of samples of commercial butter were studied by holding small portions at 21.1° C. (70° F.) for several days. A number of these developed a defect that was considered to be surface taint and this occurred when the original quality was good as well as when it was poor. The characteristic odor and flavor were very conspicuous. In most instances the butter that developed surface taint on holding was unsalted or had a low salt content, often less than 1 percent. Butter produced by churning cream into which a small portion of one of the defective samples had been inoculated, regularly developed surface taint in a few days at 15.6° C. (60° F.). In general, the defect could be produced down through a series of churnings as with commercial surface taint samples.

MICROSCOPIC EXAMINATION OF EXPERIMENTALLY PRODUCED SURFACE TAINT BUTTER

The organisms in a number of samples of experimentally produced surface taint butter were studied by direct observation. Two procedures were used, (a) smearing out the butter in a thin film on a slide and staining with Newman's solution and (b) the method employed by Hammer and Nelson (5) for the microscopic examination of butter. Excessive numbers of bacteria were regularly found, and in some instances more than half a billion per milliliter were present. The flora of the various samples differed a great deal. Micrococci were often conspicuous, but in other instances were present in only very small numbers. Rather long thin rods were noted regularly in the defective butter.

A number of samples of commercial surface taint butter were also examined microscopically and the results secured were in essential agreement with those secured on experimentally produced surface taint butter.

SIGNIFICANCE OF EXPERIMENTAL PRODUCTION OF SURFACE TAINT BUTTER

The production of surface taint through a series of churnings by the inoculation of defective butter into the cream to be churned, definitely indicates the biological nature of the defect. The development of surface taint in certain commercial samples of unsalted or low salted butter held under conditions favorable for bacterial development suggests that organisms capable of producing surface taint may be present in samples of butter which show no evidence of the defect. It would ap-

pear that when certain organisms encounter certain conditions in butter, surface taint is to be expected. The conditions necessary for growth probably include not only temperature but salt concentration and undoubtedly other factors.

ATTEMPTS TO DEVELOP SURFACE TAINT WITH CULTURES OF THE ORGANISMS PREDOMINATING IN SURFACE TAINT BUTTER

Although the predominant types of organisms secured when colonies, on beef infusion agar plates poured with surface taint butter, were picked into litmus milk did not produce an objectionable odor in the milk, attempts were made to develop surface taint with them. Many experimental lots of butter were prepared by inoculating into pasteurized — 76.7° C. (170° F.) for 10 minutes—cream, either alone or in combinations, various bacteria and yeasts that were more or less predominant on the plates poured with surface taint butter and then churning the cream at once or after it had been held for varying periods to permit the growth of the organisms; the butter was usually salted lightly. Although the organisms used produced a variety of changes in milk, the butter regularly failed to develop surface taint on holding at either 5° C. (41° F.) or 15.6° C. (60° F.). Many of the organisms produced definite changes in the odor and flavor of butter, as was evident by comparing the butter from the inoculated cream with that from uninoculated checks, but none of the large number studied gave a condition even remotely resembling surface taint. The results suggest that the organisms predominant on beef infusion agar plates poured with surface taint butter are not capable of producing surface taint in butter churned from cream into which they are inoculated.

ISOLATION OF AN ORGANISM CAPABLE OF PRODUCING SURFACE TAINT

The failure to find an organism capable of producing surface taint among those predominant on plates poured with various samples of butter showing this defect led to trials with organisms encountered only in small numbers. The general procedure was that employed with the organisms found in considerable numbers. In selecting the organisms to be studied considerable attention was given to the odor produced in milk because it seemed probable that an organism which would cause surface taint in butter would also produce an objectionable odor in milk, but the trials included many organisms producing little or no objectionable odor in milk.

A beef infusion agar plate poured with surface taint butter from Canada yielded an organism which produced typical sur-

face taint in an experimental churning of butter made from cream inoculated with it. The organism is believed to be an undescribed species and is tentatively* designated *Achromobacter putrefaciens;* a description is given in the appendix (p. 414). Additional trials with the organisms very regularly resulted in the development of surface taint in butter; milk cultures were usually used to inoculate the cream, but occasionally agar slope cultures were employed. At 15.6° C. (60° F.) the butter commonly required from 2 to 4 days for the development of surface taint, while at 5° C. (41° F.) from 7 to 10 days were required. If the defect was present to only a slight degree, holding a small portion of the butter overnight at room temperature made it very conspicuous. Small amounts of the butter that had been melted developed an extremely pronounced odor when held overnight at room temperature.

A. putrefaciens was apparently present in only small numbers in the butter from which it was isolated. The organism developed very satisfactorily on beef infusion agar slopes and, accordingly, it seemed unlikely that living cells of this type were present in the butter and failed to develop. Information on the numbers in which *A. putrefaciens* must be present per milliliter of butter to produce surface taint was secured by making plate counts (beef infusion agar incubated four days at 21.1° C., 70° F.) on several lots of butter (prepared from cream into which the organism had been inoculated) at the time surface taint developed. Results representative of those obtained are presented in table VI.

TABLE VI. NUMBERS OF BACTERIA IN BUTTER (MADE WITH *A. PUTREFACIENS*) AT THE TIME SURFACE TAINT DEVELOPED.

Holding temp. 15.6° C. (60° F.)

Plate method

Age of butter	Trial 1		Trial 2		Trial 3	
days	Odor and flavor	Bacteria per ml.	Odor and flavor	Bacteria per ml.	Odor and flavor	Bacteria per ml.
0	normal	3,000	normal	10,000	normal	10,000
2	surf. taint	42,000	surf. taint	180,000	surf. taint	900,000
6	surf. taint	440,000	surf. taint	900,000	surf. taint	290,000

The data show that *A. putrefaciens* produced surface taint in butter when the numbers per milliliter, as determined by the plate method, were comparatively small; in trial 1 surface taint was present in the butter two days old with a plate count of only 42,000 per ml. The results secured in trial 3 indicate that

*It seems probable that the genus Achromobacter will eventually be divided into several genera.

a decrease in the organisms may have occurred before a large number was present. A study of the colonies on the plates showed practically a pure culture of *A. putrefaciens*. The numbers of organisms required to produce surface taint with *A. putrefaciens* are in agreement with the idea that the type responsible for surface taint may be greatly outnumbered by species that are not particularly objectionable and, accordingly, may be difficult to find.

GENERAL CHANGES PRODUCED IN LITMUS MILK BY *A. PUTREFACIENS*

The changes produced in litmus milk by *A. putrefaciens* were of special significance because of their possible use in attempts at additional isolations. The conspicuous change was a rapid reduction of the litmus. At 21.1° C. (70° F) with a heavy inoculation this reduction sometimes occurred in as short a time as eight hours. A clearing of the milk soon began at the surface; it extended downward and became so pronounced that it very definitely was due to proteolysis. As the breaking down of the milk progressed, a putrefactive odor, that was very objectionable and like the odor of surface taint butter, developed.

ADDITIONAL ISOLATIONS OF *A. PUTREFACIENS*

The results obtained with *A. putrefaciens* led to the examination of other samples of surface taint butter for its presence. Plating on beef infusion agar and picking colonies into litmus milk after several days incubation at 21.1° C. (70° F.) failed to secure the organisms from these samples. Undoubtedly the high dilutions necessary to obtain plates that were not too crowded for picking lessened the chances of isolating it. Other isolation methods were used, including an enrichment procedure which consisted of inoculating a small amount of surface taint butter into litmus milk, holding this at 5° C. (41° F.) until reduction occurred, and then plating on beef infusion agar; often a small amount of reduced milk was transferred to a second tube, which was also held at 5° C. (41° F.), and the process continued down through a series of tubes before the plates were poured. By this enrichment method *A. putrefaciens* was isolated from five samples of surface taint butter but could not be secured from a number of other samples. It was never found in large numbers but was isolated several times from each of the five samples. The incubation of the inoculated litmus milk at 5° C. (41° F.) did not always prevent the development of rapidly-reducing, acid-forming organisms, and a type of organism which produced an unusual odor also seemed to grow rapidly at this temperature so the conditions used were not at all selective.

ATTEMPTS TO ISOLATE ORGANISMS OTHER THAN *A. PUTREFACIENS* THAT WOULD PRODUCE SURFACE TAINT

The failure to find *A. putrefaciens* in all the samples of surface taint butter examined, even when various procedures were used, led to the search for other organisms capable of causing the condition. Direct plating on beef infusion agar, followed by the picking of colonies into litmus milk, was regularly used. In another method plates were poured with beef infusion agar and, after they had solidified, the surface of one of them was smeared with a small portion of the defective butter by means of a sterile, bent glass rod; the glass rod was then used to smear a second and a third plate, the inoculating material being provided by the butter adhering to the rod. After incubating a few days at 21.1° C. (70° F.), colonies were picked into litmus milk. With this procedure great variations in the numbers of colonies, per unit of area, could be secured easily and, accordingly, there appeared to be less chance of missing an organism present in small numbers. Moreover, the fat distributed over a portion of the surface of the agar made conditions more comparable to those in butter than when the fat was absent.

Organisms other than *A. putrefaciens*, which would produce surface taint when inoculated into pasteurized cream and the cream churned, were secured from a number of samples of surface taint butter. These organisms were secured either by pouring beef infusion agar plates with the butter or by smearing surface taint butter on the surface of beef infusion agar. They were always found in small numbers, and other types which would not produce surface taint with the usual procedure of testing were the predominant ones. Studies of the organisms isolated showed that they varied considerably in their general characters.

Cultures which would produce surface taint were secured from 17 samples of commercial surface taint butter, 6 from Canada and 11 from the United States; the 6 samples from Canada were from 6 butter plants while the 11 samples from the United States were from 9 butter plants. *A. putrefaciens* was found in 6 samples of butter, 4 from Canada and 2 from the United States.

The examination of a considerable number of samples of surface taint butter failed to yield organisms capable of producing the defect. With some of these samples surface taint could be carried through a series of experimental churnings by using defective butter to inoculate the cream. Abundant growth of bacteria was secured on beef infusion agar with all of the samples but the organisms picked into litmus milk did not produce an odor suggestive of surface taint and those used in ex-

perimental butter failed to produce the defect. Plating the butter on beef infusion agar and smearing it on beef infusion agar plates were the procedures most widely used, but various other methods were employed with one or more samples. One of the methods involved making a series of dilutions of the butter in tubes of litmus milk with the object of diluting out the organisms that were unimportant. The results were unsatisfactory in that the highest dilutions showing growth usually produced acid slowly; this would be expected from the abundance of slow acid formers among the organisms picked into litmus milk from agar plates poured with defective butter. Although anaerobes did not seem a probable cause of surface taint because the defect devolops first at the surface of butter,various procedures intended to favor organisms preferring a reduced oxygen tension were used. Tubes of litmus milk, which had been boiled to drive off the oxygen and then immediately cooled in ice water, were inoculated with different dilutions of surface taint butter and the surface flooded with a mixture of vaseline and paraffin. The dilution procedure was also tried with tubes of sheep brain medium, both with and without 0.1 percent glucose; after a period of growth, beef infusion agar slope cultures were made and colonies picked into litmus milk. Plates poured with beef infusion agar were incubated in containers in which the air was partially or completeley displaced by carbon dioxide. When all of the air was displaced very few colonies developed, but colonies appeared in large numbers when the plates were held in the air.

The possibility of a filterable virus being responsible for surface taint was studied in two trials by securing serum from defective butter, filtering it through a bacteria-proof filter after the addition of sterile water to reduce the viscosity of the serum and then adding the filtrate to pasteurized cream shortly before churning; cultures made from the filtrate showed no growth. The experimental butter was held at 5° C. (41° F.) or 15.6° C. (60° F.) and examined periodically. At all stages of the storage period, which extended over several weeks, the butter made by the addition of the filtrate to the cream was as satisfactory as the controls, and there was no suggestion of surface taint.

CHARACTERS OF ORGANISMS STUDIED THAT ARE IMPORTANT FROM STANDPOINT OF THE BUTTER INDUSTRY

1. Production of surface taint. The outstanding character of the organisms studied, from the standpoint of their importance to the butter industry, is the production of surface taint in butter. When any one of them was inoculated into pasteurized cream and the cream churned, the resulting butter devel-

oped surface taint in from 2 to 4 days at 15.6° C. (60° F.) and in from 7 to 10 days at 5° C. (41° F.). Each of the organisms also produced a very disagreeable odor, not unlike that of surface taint butter, when it was grown in sterile milk for several days.

2. Influence of salt and butter culture in restraining the development of surface taint. The influence of salt and butter culture in restraining the development of surface taint was investigated by making series of churnings with the general procedure used in studying the influence of organisms on the odor and flavor of butter; the amount of salt was varied, and butter culture was used in certain of the churnings. The samples of butter in a series were all made from one lot of inoculated cream so that the inoculation with the organism causing surface taint was uniform. The butter culture was never permitted to ripen the cream, but 10 percent was added just before the churning.

Table VII gives the results obtained when surface taint butter was used as the inoculating material. The unsalted and low salted butter developed surface taint in two days while the medium salted, butter culture unsalted and butter culture salted lots remained normal during the seven-day holding period. After two days the direct counts on all the lots made from the inoculated cream were comparatively high, with the lots that had developed surface taint showing higher counts than the others. After seven days the direct counts on all the inoculated lots except one were higher than after two days,

TABLE VII. INFLUENCE OF SALT AND BUTTER CULTURE IN RESTRAINING THE DEVELOPMENT OF SURFACE TAINT.

Surface Taint Butter Used to Inoculate Pasteurized Cream

Results after holding at 15.6° C. (70° F.) for

Butter	2 days		7 days		
	Odor and flavor	Bacteria per ml. Direct count	Odor and flavor	Bacteria per ml.	
				Plate count	Direct count
Uninoc. control; normal salt	normal	1,600,000	normal	210,000	1,000,000
Unsalted	s.t.*	530,000,000	s.t.	124,000,000	660,000,000
Low salted	s.t.	430,000,000	s.t.	48,500,000	800,000,000
Medium salted	normal	100,000,000	normal	7,500,000	150,000,000
Butter culture; unsalted	normal	185,000,000	normal	8,600,000	160,000,000
Butter culture; salted	normal	110,000,000	normal	8,000,000	130,000,000

*s.t.—surface taint.

and the lots that had developed surface taint again showed higher counts than the others; the plate counts were regularly much lower than the direct counts, but those secured on the butter that had developed surface taint were higher than those on the butter having a normal odor and flavor. The butter with which butter culture was used always showed many streptococci by the direct method.

Table VIII gives the data secured when *A. putrefaciens* was used to inoculate the pasteurized cream. The unsalted and low salted lots developed surface taint in four days (there was a suggestion of the defect in three days), while the medium salted, butter culture unsalted and butter culture salted lots were still normal after 20 days. The bacterial counts, both plate and direct, showed a close relationship to the salt concentrations. When butter culture was not used the medium salted butter showed comparatively small numbers of organisms throughout the holding period; the low salted butter showed higher numbers with an increase throughout the holding period according to the direct counts and no regular change according to the plate counts; the unsalted butter showed a very high direct count after four days and then a decrease according to the direct count, while the plate count again showed no regular change. When butter culture was present the salted butter showed smaller numbers than the unsalted by both methods; there was a decrease, according to the plate method, with either type of butter, while according to the direct method there was an increase.

Table IX gives the results secured when a pure culture of a type other than *A. putrefaciens* was used to inoculate the pasteurized cream. The unsalted and low salted butter developed surface taint in 3 days, while the other lots were still normal after 11 days.

The data presented in tables VII to IX, inclusive, illustrate the great influence of salt and butter culture in restraining the development of surface taint. It is improbable that the salt percentage in the butter or the butter culture percentage in the cream which prevented the development of surface taint under the conditions of the experiments will always prevent the development of surface taint because of the various other factors which influence the growth of organisms in butter, and also because organisms other than those used are undoubtedly capable of producing surface taint, but the results are suggestive from the standpoint of controlling the defect.

3. Influence of acid in preventing the development of *A. putrefaciens* in milk. Since the addition of butter culture to cream was so effective in preventing surface taint in butter made from the cream, the influence of acid in preventing the

TABLE VIII. INFLUENCE OF SALT AND BUTTER CULTURE IN RESTRAINING THE DEVELOPMENT OF SURFACE TAINT.
A. putrefaciens Used to Inoculate Pasteurized Cream
Results after holding at 15.6° C. (60° F.) for

Butter	Composition		4 days			10 days			20 days		
	% Salt	% H_2O	Odor and flavor	Bacteria per ml.		Odor and flavor	Bacteria per ml.		Odor and flavor	Bacteria per ml.	
				Plate count	Direct count		Plate count	Direct count		Plate count	Direct count
Uninoc. control; normal salt			normal	23,000	< 600,000	normal	3,500,000	4,300,000	normal	4,600,000	14,000,000
Unsalted			s.t.*	2,270,000	800,000,000	s.t.	700,000	430,000,000	s.t.	7,500,000	425,000,000
Low salted	0.75	13.8	s.t.	180,000	75,000,000	s.t.	60,000	320,000,000	s.t.	430,000	420,000,000
Medium salted	1.5	13.2	normal	1,000	2,000,000	normal	9,000	1,500,000	normal	8,000	800,000
Butter culture; unsalted			normal	12,000,000	30,000,000	normal	6,700,000	67,000,000	normal	1,500,000	60,000,000
Butter culture; salted	0.5		normal	400,000	11,000,000	normal	100,000	18,000,000	normal	110,000	30,000,000

*s.t.—surface taint.

TABLE IX. INFLUENCE OF SALT AND BUTTER CULTURE IN RESTRAINING THE DEVELOPMENT OF SURFACE TAINT.

Pure Culture of a Type Other Than *A. putrefaciens* Used to Inoculate Pasteurized Cream

Butter	Odor and flavor after holding at 15.6° C. (60° F.) for 3 days	7 days	11 days
Uninoc. control; normal salt	normal	normal	normal
Unsalted	s.t.*	s.t.	s.t.
Low salted	s.t.	s.t.	s.t.
Medium salted	normal	normal	normal
Butter culture; unsalted	normal	normal	normal
Butter culture; salted	normal	normal	normal

*s.t.—surface taint.

development of one of the organisms causing surface taint was also studied; *A. putrefaciens* was selected because it produced surface taint so rapidly. Fifty ml. portions of sterile skimmilk in flasks were acidified by the addition of different quantities of sterile lactic acid and then inoculated with *A. putrefaciens*; after incubating five days at 21.1° C. (70° F.), each flask was cultured on a beef infusion agar slope. Preliminary trials indicated the general percentage of lactic acid required to restrain growth and, in the final trial, milk with acidities (calculated as lactic acid) of 0.27, 0.28 and 0.29 percent permitted growth, while milk with acidities of 0.30 and 0.31 percent did not.

4. Temperature relationships of the organisms isolated. The growth of the organisms at various temperatures was studied by inoculating sterile, litmus milk with actively growing cultures; changes in the appearance of the milk were used as a basis for determining growth. At 5° C. (41° F.) all the organisms grew and with *A. putrefaciens* a change in the litmus milk was noted in approximately five days; at 21.1° C. (70° F.) all the organisms grew very well, and with some of them inoculated litmus milk showed a definite change overnight; at 37° C. (98.6° F.) only 6 of the 17 cultures isolated showed any evidence of growth, and all of the *A. putrefaciens* cultures failed to grow.

5. Heat resistance. The heat resistance of seven cultures (two of *A. putrefaciens*) capable of producing surface taint was studied, using both young (about 7-hour) and old (about 96-hour) cells from agar slopes and also from milk. The cells from agar were prepared by thoroughly distributing the growth in sterile milk and the cells from milk by simply diluting a milk culture with sterile milk. The preparations were

distributed, in 2 ml. quantities, to small test tubes, the tubes sealed and the heating carried out at 61.1° C. (142° F.) in a water bath. After heating, the tubes were cooled, opened and ½ ml. portions transferred to tubes of litmus milk; observations for growth were made from time to time. None of the cultures resisted five minutes heating at 61.1° C. (142° F.), regardless of whether the cultures were young or old, or whether they came from agar slopes or from milk. With two of the cultures, the young cells from agar resisted 61.1° C. (142° F.) for three minutes but the old cells from agar and both the young and old cells from milk failed to resist this exposure.

ATTEMPTS TO PRODUCE SURFACE TAINT WITH *PS. FLUORESCENS*

Since the work of various investigators suggests that *Ps. fluorescens* may be responsible for surface taint and the closely related conditions, a considerable number of experimental churnings was made with this organism. Some of the cultures used were secured from culture collections, while others were isolated from dairy products, especially from milk that had been held at 5° C. (41° F.). The procedure usually employed was to inoculate the culture to be studied into pasteurized cream and then churn the cream at once; after it had been washed, the butter was salted lightly. With all the cultures used the butter quickly developed an objectionable condition. This may have resembled surface taint for two or three days, but soon pronounced rancidity was evident and it persisted for long periods. The rancidity developed at both 15.6° C. (60° F.) and 5° C. (41° F.).

VARIATIONS IN SURFACE TAINT

The butter submitted as examples of surface taint did not all show exactly the same condition. As has already been pointed out, the objectionable odor and flavor of a few of the samples seemed to be due to the raw material used, but distinct variations were evident in the samples which apparently developed the off odor and flavor after the manufacture of the butter. With the varied flora generally found in surface taint butter it would be expected that more than one type of organism would influence the odor and flavor of the product and that, accordingly, variations in the condition would occur. Undoubtedly with more than one type of organism capable of producing surface taint, variations would occur if, due to very unusual circumstances, the organisms were present in practically pure cultures in the butter.

The odors and flavors secured when butter containing a comparatively low salt content was held at 21.1° C. (70° F.)

showed some interesting variations. Certain of the samples developed a typical surface taint, while others showed a cheesiness of one type or another; the cheesiness varied from a condition suggesting Limburger to a typical cheddar condition, and one sample was encountered which had an odor and flavor definitely suggesting Swiss cheese. These results suggest the variations that may be expected when odors and flavors are developed in butter through the action of a mixed flora that encounters favorable growth conditions.

DISCUSSION OF RESULTS

The development of surface taint through a series of churnings, by inoculating defective butter into pasteurized cream and churning the cream, indicates the biological nature of the condition, and this was confirmed by the isolation of organisms that produced surface taint when inoculated into cream and the cream churned. The rather regular presence of large numbers of bacteria in surface taint butter is of no significance because large numbers of bacteria are present in many samples of normal butter, as well as butter showing defects other than surface taint. The failure to produce surface taint in butter with the organisms predominating on beef infusion agar plates is what would be expected from the changes produced in milk by these organisms; many of them produced acid, either with or without coagulation, and none produced objectionable odors.

The failure to develop surface taint by the inoculation of pure cultures of organisms or defective butter into normal butter, when it could be produced by inoculating these materials into cream and churning the cream, indicates that the organisms cannot spread widely through a mass of butter and is in agreement with the theory of Rahn and Boysen (10). These results are of considerable practical significance since they suggest that surface taint is not caused by contamination of the surface of a piece of butter but rather by contamination of the cream or the uncompleted butter. The organisms studied were easily destroyed by heat and, accordingly, it seems probable that proper pasteurization controls the contamination of the cream up to the time of heating and that the organisms causing surface taint gain entrance in the plant following the pasteurization of the cream.

The production of surface taint in butter by more than one species is what would be expected from a consideration of other changes produced in dairy products by microorganisms. Most of these changes can be caused by a number of species, and often the various types responsible for a certain change are not at all closely related. It appears that protein decomposition of a fairly definite character is involved in the development of

surface taint and that this can be caused by more than one species, just as with protein decomposition in other products.

The great influence of salt and butter culture on the development of surface taint by the organisms isolated is of special significance since it suggests methods of attempting to control the defect. The effect of salt is also evident from the more frequent appearance of surface taint in samples of commercial butter held at favorable growth temperatures when these were unsalted or low in salt than when the salt content was higher. It should be recognized that the various organisms capable of producing surface taint may be influenced differently by salt and also by butter culture so that conditions which control one species may not control another.

The variations that occur in the odor and flavor of surface taint butter would be expected when it is recognized that the conditions which permit the growth of the types that are primarily responsible for the defect also permit the growth of other types and that some of the latter may have an effect on the odor and flavor of the butter. The objectionable odors and flavors that are sometimes present in butter as the result of very poor cream may suggest surface taint, but they represent a distinctly different set of conditions. It does not appear probable that the same organisms are involved in the two types of changes since the organisms found capable of producing surface taint in butter are sensitive to acid, and the development of acid is commonly the first change that occurs in the fermentation of cream.

The prominence of surface taint in recent years is probably due to the great changes that have been introduced in the methods of manufacturing butter. Certain of these changes are very definitely traceable to studies on the factors influencing butter deterioration, while others are the result of a shift in the market demands, due to a preference on the part of certain consumers for butter having a mild flavor and also to the use of butter in the making of ice cream. The changes in the manufacturing methods have largely eliminated some of the serious flavor and aroma defects of butter. The control of certain types of deterioration would be expected to permit the occasional appearance of others that are favored by the new conditions, or that were inconspicuous in the presence of a more serious type. In general, it would be expected that the elimination of such conditions as high acidity in the cream, which apparently accelerate certain chemical changes, would favor the growth of bacteria and, accordingly, the deterioration that accompanies the development of certain species. The use of only small percentages of salt or none at all would also greatly favor bacterial deterioration.

SUGGESTED METHODS FOR THE CONTROL OF SURFACE TAINT

The studies that have been made of surface taint butter, although by no means complete, suggest methods that may be of importance in the control of the defect. The heat resistance tests on the organisms found capable of producing the condision showed that the common pasteurizing temperatures will destroy them. This indicates that, with efficient pasteurization, there must be sources of the organisms around the plant.

Creamery sanitation is of extreme importance with any microbiological defect of butter. The various pieces of equipment with which cream and butter come in contact during the process of butter manufacture afford opportunities for contamination that are certain to be serious unless satisfactory methods of cleaning and sterilizing are used. While there are no data indicating that the churn is a source of the organisms capable of causing surface taint, it has been recognized as an important source of organisms generally and, accordingly, should be considered in connection with this defect. The materials used and the nature of the construction are evidence against it.

Various investigators have suggested the relationship of polluted water to surface taint in butter. Although information on the sources of the organisms found capable of producing surface taint is not available, their general characters suggest a relationship to certain of the water and soil forms.

The percentage of salt is very important in the control of microorganisms in butter and, undoubtedly, has a relationship to the development of surface taint. Most surface taint butter has been found to have a comparatively low salt content. In the trials made with two of the organisms isolated, surface taint did not appear in butter made from inoculated cream when the salt content was 1.5 percent; it should be noted that in these trials the moisture content was low. The present demand for unsalted and low salted butter may be expected to result in more difficulty from various types of bacterial deterioration.

The value of butter culture in the control of surface taint should be recognized. When such a culture is employed, either with or without actual ripening of the cream, the number of butter culture organisms in the fresh butter is comparatively high. The changes in the number of butter culture organisms in butter are greatly influenced by the salt content but under any condition the organisms would be expected to influence the development of the types found capable of producing surface taint, since these belong to a general group of organisms that is sensitive to acid.

Temperature is an important factor in the control of bacterial growth in butter as well as in other materials, and low temperatures may prevent deterioration in butter so heavily contaminated that it would undergo rapid changes at higher temperatures. It is to be expected, however, that butter will sometimes encounter comparatively high temperatures in transit, in retail channels, in the homes of consumers, etc., and this emphasizes the necessity of manufacturing butter so that it does not contain organisms capable of causing rapid deterioration under any conditions. While the low temperatures used for holding butter have undoubtedly often prevented the development of bacterial defects, dependence on holding temperatures alone is inadvisable because of the possibility of the butter unexpectedly encountering higher temperatures; moreover, the tendency to use lower salt percentages with butter reduces the restraining action of the common holding temperatures. The object should be to manufacture butter under such careful conditions that even if it is exposed, for a short time, to temperatures favorable for the growth of organisms there will be no danger of serious bacterial deterioration.

It should be recognized that with different types of organisms capable of producing surface taint, variations in the restraining action of such factors as salt, butter culture and temperature are to be expected. A salt concentration or a temperature which effectively prevents the development of one type of organism may fail with another, and butter culture may have a restraining action on one species and not on another. Moreover, it seems probable that a combination of restraining factors will prevent the development of surface taint when only one of the factors will not. The number of organisms per milliliter of the butter is undoubtedly also of importance; with a given set of conditions the development of surface taint may occur when comparatively large numbers of organisms are present but not with only small numbers.

APPENDIX

DESCRIPTION OF *ACHROMOBACTER PUTREFACIENS*

The following description of *A. putrefaciens* applies primarily to five of the six cultures isolated. One of the cultures showed a few minor variations which are detailed at the close of the description.

MORPHOLOGY

Form and size—Rods; the cells in a young beef infusion agar culture varied from 0.5 to 1.0 by 1.1 to 4.0 microns and averaged about 0.8 by 2.5 microns.

Arrangement—Singly and in pairs in preparations from agar or milk.

Motility—Actively motile; flagellation monotrichous.

Staining reaction—Stains readily with the usual stains; gram negative.

Spore formation—Spores never observed; the organism was easily destroyed by heat.

CULTURAL CHARACTERISTICS

Agar slope—Growth on beef infusion agar slopes was echinulate, slightly reddish-brown and somewhat viscous in 24 hours at 21° C. Growth increased greatly with age and became more distinctly reddish-brown. There was also abundant growth on the agar which is standard for milk analysis.

Agar stab—Beef infusion agar stabs showed a heavy, reddish-brown, smooth-edged, somewhat viscous surface growth and an echinulate, white growth along the line of inoculation in 24 hours at 21° C. Growth increased greatly with age, and the color in the surface portion became more distinctly reddish-brown.

Agar colony—Colonies were evident on beef infusion agar plates at 21° C. in 24 to 48 hours. Well developed surface colonies were round, shiny, smooth-edged, slightly raised, somewhat transparent, with a brown tinge and from 1 to 4 mm. in diameter; under the microscope the structure appeared granular except at the edge. Well developed subsurface colonies were irregular in shape, with many approximately oval, and the diameter was from ¼ to 1 mm.; under the microscope the structure appeared granular.

Gelatin stab—Growth was evident in 24 hours at 21° C. and liquefaction was evident in 48 hours. Liquefaction progressed rapidly and was soon saccate to stratiform, with a turbidity and a reddish-brown sediment in the liquefied portion.

Bouillons—Bouillons showed a turbidity and later a thin, grey, easily broken pellicle. A sediment developed rather rapidly. Eventually the sediment had a reddish-brown color.

Dunham's Solution—In Dunham's solution there developed a turbidity, a thin pellicle which was easily broken and eventually a sediment; the sediment became reddish-brown with age.

Uschinsky's Solution—In Uschinsky's solution there was a turbidity, a thin pellicle which was easily broken and finally a sediment; commonly the sediment developed a reddish-brown color.

Potato—Growth on potato was echinulate, shiny, somewhat viscous and reddish-brown, with the color especially pronounced in old

cultures. Ordinarily growth was comparatively slow but eventually abundant.

Litmus milk—The first change in litmus milk was a rapid reduction which began at the bottom; in some instances reduction was evident in 8 hours at 21° C. After complete reduction, proteolysis began at the surface and continued until the milk was very largely digested. In young milk cultures there was a putrefactive odor while in old cultures the odor was objectionable but not putrefactive.

Plain milk—Aside from the litmus reduction, the changes in plain milk were essentially like those in litmus milk.

BIOCHEMICAL CHARACTERISTICS

Gas production—Gas production not observed.

Indol—Not produced.

Acetylmethylcarbinol from glucose—Not produced.

Nitrates—Rapidly reduced to nitrites.

Ammonia—Rapidly produced.

Reaction change—In bouillons acid was produced from maltose and sucrose but not from glycerol, arabinose, galactose, glucose, levulose, lactose, mannitol, sorbitol, salicin, raffinose, dextrin, inulin or starch.*

GROWTH CONDITIONS

Oxygen relationship—Facultative; grew very well aerobically.

Growth temperature—Grew well at 21° C.; considerably slower at 5° C. No growth at 37° C.

One of the six cultures studied showed minor variations from the above description. With it the digestion of milk was less rapid, gelatin was liquefied comparatively slowly and acid was produced from glucose.

*With rapid production of ammonia it is quite possible that slow acid production may be missed.

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